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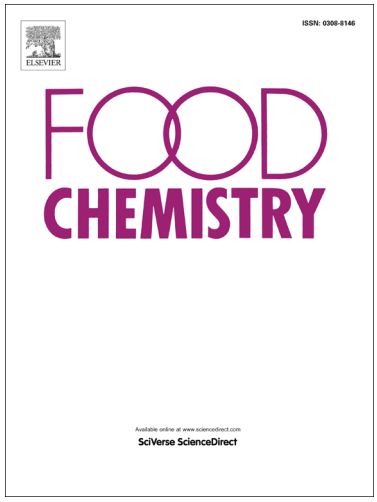

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Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials

Running title: Encapsulation of antioxidant phenolics extracted from spent coffee

Lina F. Ballesteros [a], Monica J. Ramirez [b], Carlos E. Orrego [b], José A. Teixeira [a], Solange I. Mussatto [c,*]

[a] *Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal*

[b] *Instituto de Biotecnología y Agroindustria, Universidad Nacional de Colombia sede Manizales. Km 7, via al Magdalena, Campus La Nubia, Manizales, Colombia*

[c] *Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark*

* Corresponding author at: Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet, Building 220, 2800, Kongens Lyngby, Denmark. Tel.: +45 93 51 18 91. *E-mail addresses:* smussatto@biosustain.dtu.dk, solangemussatto@hotmail.com (S.I. Mussatto).

ABSTRACT

Freeze-drying and spray-drying techniques were evaluated for encapsulation of phenolic compounds (PC) extracted from spent coffee grounds. Additionally, the use of maltodextrin, gum arabic and a mixture of these components (ratio 1:1) as wall material to retain the PC and preserve their antioxidant activity was also assessed. The contents of PC and flavonoids (FLA), as well as the antioxidant activity of the encapsulated samples were determined in order to verify the efficiency of each studied condition. Additional analyses for characterization of the samples were also performed. Both the technique and the coating material greatly influenced the encapsulation of antioxidant PC. The best results were achieved when PC were encapsulated by freeze-drying using maltodextrin as wall material. Under these conditions, the amount of PC and FLA retained in the encapsulated sample corresponded to 62% and 73%, respectively, and 73-86% of the antioxidant activity present in the original extract was preserved.

Keywords: Spent coffee grounds; Encapsulation; Freeze-drying; Spray-drying; Phenolic compounds; Antioxidant activity

1. Introduction

Phenolic compounds are secondary metabolites synthesized by many plants during their normal development or as a response to environmental stress conditions (Beckman, 2000). These compounds present important functional properties being, therefore, of great interest for chemical, pharmaceutical and food industries. In green coffee, phenolic compounds have been mainly identified as chlorogenic acid and related to substances including caffeoylquinic acid, dicaffeoylquinic acid, feruloylquinic acid, and p-coumaroylquinic acid, which are partially transformed during the coffee roasting process (Farah & Donangelo, 2006; Mussatto, Machado, Martins & Teixeira, 2011a). Numerous benefits for the health have been reported as a consequence of the ingestion of phenolic compounds present in coffee (Mussatto, 2015), particularly for chlorogenic acid, including antioxidant activity and anti-obesity (Cho et al., 2010), anti-inflammatory (Shin et al., 2015), anti-diabetic (Karthikesan, Pari & Menon, 2010) and anti-cancerous effects (Kasai, Fukada, Yamaizumi, Sugie & Mori, 2000).

However, phenolic compounds are very vulnerable to an oxidizing environment, for example, to light, oxygen, moisture, among others, due to the existence of unsaturated bonds in the molecular structures. Thus, they must be encapsulated to enhance their storage stability, making safer as food ingredients and providing benefits to the consumers. Apart from stabilizing these bioactive compounds, the encapsulation process also helps to mask unpleasant flavours in food provided by these functional compounds, including bitter taste and astringency of polyphenols. A large variety of materials can be used for encapsulation in food applications, being polysaccharides such as maltodextrin, gum arabic, hydrophobically modified starches and chitosan, as well as mixtures of them, the most commonly used coating materials (Gouin, 2004; Nedovic, Kalusevic, Manojlovic, Levic & Bugarski, 2011; Ray, Raychaudhuri & Chakraborty, 2016).

Encapsulation techniques are often based on drying processes due to the liquid nature of the extracts that contain the bioactive compounds. Spray-drying, spray-bed-drying, fluid-bed coating and freeze-drying are some examples of encapsulation techniques. Among these technologies, spray-drying is one of the most widely used for food industry due to its low-cost and flexibility (Fang & Bhandari, 2010), together with freeze-drying, which is very suitable for drying of heat sensitive compounds since it conserves almost intact the initial functional properties of those components (Ceballos, Giraldo & Orrego, 2012). However, the drying technique and the material used as coating usually affect the retention capacity of compounds within the matrix. Therefore, it is of great importance to properly select both, the coating material and the encapsulation technique in order to maximize the incorporation and retention of the functional compounds within the encapsulation matrix. Maltodextrin, for example, is a relatively low-cost polysaccharide with neutral taste and aroma and that acts as an effective protection to flavours (Fernandes, Borges & Botrel, 2014). Gum arabic is also a polysaccharide commonly used in encapsulation processes due to its good emulsifying and film-forming capacities (Silva et al., 2013). Several researchers have studied the use of these two coatings -unmixed and mixed- to encapsulate bioactive compounds such as essential oils (Fernandes et al., 2014), anthocyanins (Flores, Singh, Kerr, Pegg & Kong, 2014; Mahdavee Khazaei, Jafari, Ghorbani & Hemmati Kakhki, 2014), cherry pomace phenolic extracts (Cilek, Luca, Hasirci, Sahin & Sumnu, 2012), propolis (Silva et al., 2013), among others, being the retention capacity highly dependent on the type of phenolic compound encapsulated and on the coating composition.

Most of the bioactive compounds that are encapsulated into these matrices have been extracted from natural sources. Spent coffee grounds (SCG), which is the main residue of coffee industry obtained from soluble coffee preparation (Mussatto et al., 2011a), has attracted an increased interest as source of bioactive compounds, specially due to its high content of

phenolic compounds (Ballesteros, Ramirez, Orrego, Teixeira & Mussatto, 2017; Conde & Mussatto, 2016; Murthy & Naidu, 2012; Mussatto, Ballesteros, Martins & Teixeira, 2011b; Panusa, Zuorro, Lavecchia, Marrosu & Petrucci, 2013; Zuorro & Lavecchia, 2012). However, the encapsulation of these compounds for the maintenance of their properties has never been reported. Phenolic compounds extracted from SCG present important properties like antioxidant activity, for example, which make possible their application in different areas. Encapsulation of these compounds is an important strategy to preserve their properties for longer periods since the phenolic compounds would be protected from oxidation by the coating material that acts as a barrier to oxygen and water, improving their stability and use as a food additive (Lavelli, Harsha, & Spigno, 2016). In this sense, the present study evaluated the encapsulation of antioxidant phenolic compounds extracted from SCG by using two different encapsulation techniques, namely freeze-drying and spray-drying. The efficiency of maltodextrin, gum arabic and a mixture of these components as wall material to retain the phenolic compounds and preserve their antioxidant activity within the encapsulated matrix was also evaluated.

2. Materials and methods

2.1. Raw material and chemicals

Spent coffee grounds (SCG) were provided by the Portuguese coffee industry Nova Delta-Comércio e Indústria de Cafés S.A. (Campo Maior, Portugal). The material was dried in an oven at 60 ºC until 5% moisture content being then stored for further use in the extraction experiments. All the chemicals used were analytical grade. Maltodextrin (dextrose equivalent

20 (DE20)) and gum arabic were purchased from Sigma–Aldrich (Chemie GmbH, Steinheim, Germany). Ultrapure water from a Milli-Q System (Millipore Inc., USA) was used.

2.2. Extraction procedure

The extraction of antioxidant phenolic compounds from SCG was performed by autohydrolysis using the conditions optimized in a previous study (Ballesteros et al., 2017). Briefly, ultrapure water and SCG (15 ml/g) were mixed into 160-ml cylindrical stainless steel reactor (Parr Instruments Company, Illinois, USA), which was duly closed and placed into an oil-bath with open heating circulator and temperature control (Julabo, Labortechnik GmbH, Seelbach, Germany). The reactor was maintained in the bath for 50 min at 200 °C, being subsequently removed and immediately cooled down in an ice-bath for 10 min to stop the reaction. The total content of the reactor was centrifuged (2500 *g*, 20 min) and the supernatant (SCG extract) was filtered through 0.22 µm filters and stored at -20 ºC until further use. The volume of extract recovered after centrifugation was quantified and used for calculations.

In order to evaluate the structural properties of the extracted phenolic compounds, SCG extract was submitted to a reaction for the phenolic compounds precipitation. In brief, the extract was mixed with ethyl acetate (1:3 v/v) and the mixture was kept at room temperature during 24 h, being then centrifuged (2500 *g*, 20 min) and the precipitated dried at 100 °C.

2.3. Encapsulation process

Encapsulation of the SCG extract was carried out using maltodextrin and gum arabic as coating materials. For the assays, 100 ml of extract were mixed with 20 g of coating material and the mixture was homogenized at 6000 rpm in an IKA T-25D Ultra-turrax homogenizer

until obtaining a good dispersion. Three matrices were evaluated: i) 100% maltodextrin; ii) 100% gum arabic; and iii) a mixture of maltodextrin and gum arabic at ratio 1:1. A blank consisting of distilled water instead of SCG extract was also prepared for each matrix. All the samples were prepared in triplicate and the total soluble solids (°Brix) were measured using a digital refractometer. Afterward, the samples were subjected to freeze-drying and spray-drying processes. For freeze-drying, the samples were previously frozen and then put into a chamber at -60 °C under pressure of 0.05 bar, being maintained under these conditions for 48 h. A Christ alpha 1-4 LD equipment (SciQuip, UK) was used. Spray-drying was carried out in an equipment mini Buchi model 191 (Büchi Laboratoriums Technik, Switzerland) using a liquid feed volumetric flow rate of 108 ml/h, drying air inlet temperature of 100 °C, nozzle air flow-rate, 600 NL (litters at normal conditions)/h and aspiration 75% (28 m^3/h).

The moisture content of the dry powders was determined in a moisture analyser model MAC 50/1/NH (Radwag, Poland) and they were stored at room temperature and protected from the light until further analyses.

2.4. Analytical methodology

2.4.1. Chemical characterization of SCG extract

High performance liquid chromatography was used to analyse the compounds present in the SCG extract. Chlorogenic acid, hydroxymethylfurfural, and furfural were identified and quantified in the extract using the following conditions (Mussatto et al., 2011b): UV detector at 276 nm and a Nucleosil 120-5 C18 5 µm (4.6 × 250 mm) column at room temperature. Acetonitrile/water (ratio 1/8) with 10 g/l of glacial acetic acid (pH adjusted to 2.5 with phosphoric acid) was used as mobile phase at 0.9 ml/min. The responses of the detector were integrated using the D-7000 HPLC System Manager software (Hitachi).

174

175 *2.4.2. Structural characterization*

176 Morphology and crystalline phases of SCG extract and encapsulated phenolic
177 compounds were evaluated by scanning electron microscopy (SEM) and X-ray diffraction
178 (XRD), respectively (Ballesteros, Teixeira & Mussatto, 2014a). For the SEM analyses, the
179 samples were covered with a very thin film (35 nm) of Au-Pd (80–20 wt.%) and the images
180 were obtained by applying an acceleration voltage of 10 kV. For the XRD analyses, the
181 radiation was generated at 25 mA and 35 kV. The scattering angle of 2θ from 10° to 100° was
182 measured at the step size of 0.04 and 1 s exposure at each step.

183 Chemical groups and bonding arrangement of constituents present in the samples were
184 determined by Fourier transform infrared spectroscopy (FTIR) using a Perkin- Elmer 16 PC
185 spectrometer (Boston, USA) equipped with a diamond-composite attenuated total reflectance
186 (ATR) cell. The measurements were recorded with a wavenumber range from 4000 to 400 cm^{-1}
187 and 16 scans per sample. Differential scanning calorimetry (DSC) and thermogravimetric
188 analyses (TGA) were carried out as described by Ballesteros et al. (2014a). Briefly,
189 approximately 10 mg of the sample were placed in an aluminium pan and an empty pan was
190 used as a reference. The measurements were carried out between 25 and 600 °C with a linear
191 increase of 10 °C/min, under a nitrogen atmosphere.

192

193 *2.4.3. Antioxidant phenolic compounds characterization*

194 In order to evaluate the contents of phenolic compounds and flavonoids, as well as the
195 antioxidant activity of the samples after encapsulation process, the powders obtained by
196 freeze-drying and spray-drying were rehydrated until achieving the same content of soluble
197 solids measured before drying. The rehydration was calculated by using the Eq (1), where *W,*

198 is the mass of powder to hydrate; M, is the moisture of the sample after the drying process;
199 and B, represents the content of soluble solids (°Brix) in the sample before drying.
200

$$H_2O_{rehydration=} \frac{W\left(1-\frac{M}{100}\right)}{B} * 100 - \left(\frac{W*M}{100}\right) \quad (1)$$

201

202 The content of phenolic compounds (PC) in the encapsulated samples was determined
203 by using the Folin-Ciocalteu colorimetric method adapted to a 96-well microplate, as
204 described by Meneses, Martins, Teixeira & Mussatto (2013). Briefly, 5 µl of the filtered
205 sample were mixed with 60 µl of sodium carbonate solution (7.5% w/v) and 15 µl of Folin–
206 Ciocalteu reagent. Then, 200 µl of distilled water were added and the solutions were mixed
207 and heated at 60 ºC for 5 min, being subsequently cooled to room temperature and the
208 absorbance measured at 700 nm. The blank corresponding to each encapsulated was used for
209 correcting the final content of phenolic compounds in the samples. The total content of
210 phenolic compounds was expressed as milligram gallic acid equivalent per 100 ml of
211 encapsulated sample (mg GAE/100 ml).
212 The content of flavonoids (FLA) was estimated by colorimetric assay as described by
213 Meneses et al. (2013). Briefly, 30 µl of the sample was sequentially added to 90 µl methanol,
214 6 µl aluminium chloride (10% w/v), 6 µl potassium acetate (1 mol/l), and 170 µl distilled
215 water, in a 96-well microplate. The mixtures were maintained during 30 min in the dark at
216 room temperature, and the absorbance was then measured at 415 nm. The blank
217 corresponding to each encapsulated was used for correcting the final content of flavonoids in
218 the samples. The content of flavonoids was expressed as milligram quercetin equivalent per
219 100 ml of encapsulated sample (mg QE/100 ml).
220 The antioxidant activity of the encapsulated compounds was determined by the ferric
221 reducing antioxidant power (FRAP) assay as reported by Ballesteros, Teixeira & Mussatto

(2014b), and by the total antioxidant activity (TAA) assay as described by Ballesteros, Cerqueira, Teixeira & Mussatto (2015). The blanks of the encapsulated were used for correcting the final antioxidant activity of the samples. The FRAP values were expressed as millimoles of ferrous ion equivalent per 100 ml of encapsulated sample (mmol Fe(II)/100 ml), while TAA was expressed as milligrams of α-tocopherol equivalent per 100 ml of encapsulated sample (mg TOC/100 ml).

2.5. Statistical analysis

Statistical analyses were carried out using GraphPad Prism (version 6.1). One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were performed to determine the significant differences ($p < 0.05$) between the encapsulated samples.

3. Results and discussion

3.1 Extract characterization

3.1.1. Chemical composition and antioxidant activity

The contents of phenolic compounds and flavonoids, as well as the antioxidant activity values of the SCG extract before and after encapsulation, are shown in Table 1. HPLC analyses (Fig.1a) revealed also the presence of chlorogenic acid (19.99 ± 3.56 mg/100 ml) and sugar derived compounds, namely furfural (12.44 ± 2.29 mg/100 ml extract) and hydroxymethylfurfural (HMF) (18.57 ± 3.32 mg/100 ml extract) in SCG extract.

Chlorogenic acid considered the most important phenolic compound in coffee, is known to have antioxidant capacity and numerous biofunctionalities (Mussatto, 2015). Furfural is

used as an additive in food. Additionally, furfural has been identified in fruits, vegetables, beverages, bread and bread products. High furfural concentrations have been reported, for example, in cocoa and coffee (55–255 ppm) [mg/kg], wheat bread (0.8–14 ppm), cognac (0.6–33 ppm), rum (22 ppm), malt whisky (10–37 ppm), and port wine (2–34 ppm). In juices, furfural is usually found in concentrations between 0.01–4.93 ppm (Scientific Committee on Consumer Safety – SCCS, 2012). Although it is found naturally in many foods, furfural is reported to be toxic with an LD_{50} of 65 mg/kg bw (acute oral toxicity). The total potential daily per capita intake of furfural and precursors of furfural (i.e. furfuryl alcohol and furfuryl esters) from consumption of foods in which they occur naturally is approx. 0.3 mg/kg bw per day (i.e. about 300 µg/kg bw per day) in US. Thus, the intake of furfural and furfuryl derivatives from use as flavouring substances represent 1-3% of the total intake (International Programme on Chemical Safety – IPCS, 1999). Coffee presents high levels of furfural and the value found for furfural in the present study was into the allowed levels when compared with coffee values. High quantities of HMF are also found naturally in coffee. Several types of roasted coffee can contain between 300 - 2900 mg/kg of this compound (Murkovic & Pichler, 2006), being a product with very high relevance in terms of levels of HMF and quantities consumed. In the present study, the amount of HMF in the final product will depend on the food in which the encapsulated phenolic compounds will be tested. HMF is practically absent in fresh food, but it is naturally generated in sugar-containing food during heat-treatments like drying or cooking. Along with many other flavours and substances related with the colour, HMF is formed in the Maillard reaction as well as during caramelization. In these foods, it is also slowly generated during storage (Arribas-Lorenzo & Morales, 2010). Therefore, the amount of HMF in the final product must be determined considering the amount of HMF in the encapsulated extract and the HMF present in the food where the encapsulated phenolic compounds will be incorporated.

As a whole, the high content of phenolic compounds (with presence of flavonoids and chlorogenic acid) and the antioxidant activity of SCG extract confirm the great potential of SCG as a natural source of antioxidant phenolic compounds.

3.1.2. Structural characteristics

The crystallinity and chemical groups and bonding arrangement of constituents present in SCG extract after precipitation with ethyl acetate were evaluated through XRD and FTIR. The XRD pattern (Fig.1b) revealed a mostly amorphous structure. However, a broadband was diffracted around $2\theta = 20°$, revealing the existence of small crystalline regions in the SCG extract structure (Ballesteros et al., 2014a). Although the autohydrolysis process is more suitable to extract antioxidant phenolic compounds and hemicelluloses from lignocellulosic materials (Conde & Mussatto, 2016), the high temperature and extraction time (200 °C, 50 min) used during the process allowed extracting a small part of crystalline cellulose, as evidenced in Fig.1b.

The FTIR spectrum (Fig.1c) showed the typical band from 1500 to 1700 cm^{-1} ((C=O) asymmetrical and symmetric stretching vibrations) highly associated with chlorogenic acid and caffeine (Ribeiro, Salva & Ferreira, 2010) and deformation in lignin (Pandey & Theagarajan, 1997). Thus, the peak at 1654 cm^{-1} can be attributed to the absorption of these compounds, being the peak more intense when their concentration in the sample increases. The peak at 2930 cm^{-1} was assigned to the C-H_2, *n*C-H_3 stretch, being closely related to aromatic compounds with phenyl bonds similar to those in polyphenolic compounds, such as flavonoids (Mehanna, Hassan, El-Din, Ali, Amarowicz & El-Messery, 2014; Santiago-Adame et al., 2015). Supplementary bands were found in the SCG extract, being in agreement with the findings reported a previous study (Ballesteros et al., 2014a).

297 *3.1.3. Thermal behaviour*

298 DSC and TGA curves of the extract obtained by autohydrolysis of SCG and
299 subsequently precipitated with ethyl acetate are shown in Fig.1d. When the extract was
300 exposed to heating until 600 °C three events were identified. The first one revealed an
301 endothermic peak at 93.91 °C with an associated enthalpy change of 82.18 J/g, which was
302 related to the presence of impurities in the sample and vaporization of water (indicating the
303 presence of hydrophilic groups). The second event corresponded to a broad exothermic
304 transition starting at approx. 180 °C and finishing at 320 °C, accompanied with an enthalpy
305 change of 44.25 J/g. In the initial phase (180 – 256 °C) this event was related to the
306 degradation of antioxidant phenolic compounds (Reda, 2011) and in the last phase (256 – 320
307 °C) it was associated to the depolymerisation and branching of carbohydrates present in the
308 SCG extract (Ballesteros et al., 2015). Finally, the third stage started over 400 ºC and was
309 related to the decomposition of the material.
310
311 *3.2. Encapsulated samples characterization*
312
313 *3.2.1. Morphology*

314 Images obtained by SEM for the pure coating materials, as well as for the samples
315 encapsulated by freeze-drying and spray-drying techniques are shown in Fig. 2. Both coatings,
316 maltodextrin and gum arabic, possess similar morphologies. However, maltodextrin revealed
317 spheres of around 30 µm of diameter or smaller, while gum arabic showed more irregular
318 particle sizes. These spherical capsules are used to absorb the extract and, after the drying
319 process, they allow the components to remain in the coating materials. Morphology, shape,
320 and size of the capsules were expected to change after the freeze-drying and spray-drying
321 processes, due to the conditions used in each process. For spray-drying, for instance, which

utilized a temperature of 100 °C, the maltodextrin and gum arabic maintained the spherical form with very similar sizes (less than 30 µm), but in most of the cases a dehydrated aspect was shown. This morphology has been reported for spray-drying process (Santiago-Adame et al., 2015). Freeze-drying, on the other hand, clearly modified the original morphology of the coating materials, leaving a more sawdust-like morphology, both in maltodextrin and in gum arabic, typical of lyophilisation process in these matrices (Mahdavee Khazaei et al., 2014). Such morphological changes are expected to alter the power of encapsulation, due to the variation in the surface area of the coatings that allow more or less degradation of the encapsulated compounds.

3.2.2. Structural characteristics

3.2.2.1. Crystallinity and chemical bonding of constituents

Fig. 3a displays the XRD patterns for maltodextrin and gum arabic, as well as the spectra for the SCG extract encapsulated into these matrices dried by freeze-drying and spray-drying. The XRD of the samples revealed a very low degree of crystallinity, evidencing a very broad peak around $2\theta = 18°$ and an amorphous background from the beginning of the spectra to $2\theta = 55°$. Quantifying the degree of crystallinity of a compound is difficult since very small crystalline regions give broad peaks, and larger crystalline regions translate in better-defined peaks; however, the amount of such regions cannot be directly quantified. As a result, only a tendency regarding the sizes of the crystalline regions can be given. For that purpose, the peaks were fitted using a Voight function and the full width at half maximum (FWHM) was reported in the spectra in order to analyse possible differences between the samples. For larger FWHM, smaller ordered regions were expected and vice versa. Maltodextrin, for instance, showed larger FHWM compared to gum arabic, suggesting a less ordered structure. The same behaviour was kept in the samples after encapsulating the phenolic compounds regardless of

the type of drying, and when a combination of both matrices, maltodextrin, and gum arabic (ratio 1:1) was used, intermediate crystalline sizes were observed. This clearly evidences that the used coatings are the main responsible for the final structure of the encapsulated products.

FTIR results (Fig. 3b) show the predominant effect of both matrices, maltodextrin, and gum arabic, in the final sample, since the coating material structures were not affected by the addition of the SCG extract. The absorption bands typical for maltodextrin (Castro-Cabado, Casado & San Román, 2016; Santiago-Adame et al., 2015) and gum arabic (Leonor, Gómez, Kinoshita, Calandreli, Tfouni & Baffa, 2013; Paulino, Guilherme, Mattoso & Tambourgi, 2010) are summarized in Table S1 (supplementary data). It must be also stressed that the conditions used for the different drying processes did not alter the structure of the matrices since independently of the process, no significant changes are observed.

3.2.2.2. Thermal stability

DSC and TGA analyses for pure maltodextrin and gum arabic, and for the samples of SCG extract encapsulated using these coating materials were carried out in order to evaluate the thermal stability of the samples (Fig. 4). As it can be seen, the structural features exposed in the thermal characterization were largely dependent on the wall material, evidencing thus that the changes suffered in the samples are directly related to the transition temperatures of the maltodextrin and gum arabic. The first event occurring between 25 – 180 °C revealed an endothermic peak around 80 °C, which was associated with water evaporation and chemisorbed water through hydrogen bonds. This event was observed for all the samples by both, DSC and TGA analyses. Maltodextrin and the samples encapsulated with this carbohydrate presented a double peak between 190 – 360°C, generating a total weight loss of about 64%. This double transition is in agreement with the results reported by Paini, Aliakbarian, Casazza, Lagazzo, Botter & Perego (2015) and Saavedra-Leos, Leyva-Porras,

Araujo-Díaz, Toxqui-Terán & Borrás-Enríquez (2015). However, it has also been shown that the onset of this peak (~ 190 °C) can vary slightly depending on the dextrose equivalent amount that the maltodextrin possess and the water activity in which the coating and the encapsulated samples were stored (Paini et al., 2015; Saavedra-Leos et al., 2015). The second part of the maltodextrin transition coincided with the transition observed for gum arabic and the samples coated with this wall material revealed an exothermic peak, for all the samples, at about 300 °C. This transition located between 190 and 370 °C was attributed to the depolymerisation of the materials. Additionally, the samples containing gum arabic presented a weight loss of approx. 55% in this transition. Enthalpy changes and the information about peaks are shown in Table S2 (supplementary data).

Although the thermal transition indicating the decay of the samples was very close between all of them, a slight increase in the temperature was observed for the samples after encapsulation, when compared to the SCG extract without encapsulating (Fig.1d), revealing thus more thermally stable samples, mainly those encapsulated with gum arabic. This effect was more marked from the onset temperature in which the thermal degradation of the SCG extract started at lower temperatures (~ 190 °C) than those reported for the encapsulated samples with gum arabic (> 225 °C) and maltodextrin (> 190 °C), confirming that the thermal stability achieved by the encapsulated samples is provided by the material used as coating.

3.3. Encapsulation efficiency

In this step, the efficiency of the different drying processes (freeze-drying and spray-drying) and coatings to encapsulate the antioxidant phenolic compounds extracted from SCG was evaluated and compared. Fig. 5 shows the percentage of phenolic compounds and flavonoids retained within the matrix, and the antioxidant activity of the samples after

encapsulation, when compared to the initial values present in SCG extract. The results revealed that the coating used for encapsulation had an important role in the retention of antioxidant phenolic compounds within the matrix. The best results were achieved when using 100% maltodextrin as wall material and freeze-drying as encapsulation technique. Under these conditions, the amount of phenolic compounds and flavonoids retained in the encapsulated sample corresponded to 62% and 73%, respectively. These results are in agreement with those reported by Ramírez, Giraldo & Orrego (2015), where the highest content of phenolic compounds was attained when the compounds were subjected to freeze-drying and 100% maltodextrin was used as wall material. Gum arabic retained the lowest amount of phenolic compounds independently of the drying process employed. This behaviour may be explained by the fact that the encapsulation efficiency is highly dependent on the encapsulated compounds and the coating material used (Rosa et al., 2014). The antioxidant activity was expected to be reduced when compared to the initial antioxidant capacity of the SCG extract, due to the lower amount of phenolic compounds and flavonoids present in the encapsulated sample. Additionally, the reduction percentage of TAA values obtained for the matrices containing 100% maltodextrin and 100% gum arabic, presented a direct correlation with the amount of phenolic compounds retained, independently of the drying process (linear correlation, $R^2 = 0.99$). However, the lowest TAA values were observed when maltodextrin and gum arabic were mixed, indicating a detrimental effect by combining both matrices with respect to the antioxidant activity.

The drying process demonstrated to be fundamental in the efficacy of encapsulation, being freeze-drying a more effective technique for the phenolic compounds and flavonoids encapsulation. The behaviour may be partially attributed to the changes in morphology caused by the drying process. For the lyophilisation process, the sawdust-like shape creates a lower surface area/volume ratio compared to the microspheres of the spray-drying process, which

422 due to the smaller sizes of the spheres possess larger surface area for the same amount of
423 materials, allowing the phenolic compounds and flavonoids of the surface to deteriorate.
424
425 **4. Conclusion**
426
427 The technique (freeze-drying or spray-drying) and the coating material (maltodextrin,
428 gum arabic, or a mixture of these components) are factors of great influence on the
429 encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds.
430 Although gum arabic was more thermally stable when compared to maltodextrin, the
431 encapsulation with gum arabic showed a detrimental effect on the retention of phenolic
432 compounds and flavonoids, as well as on the antioxidant activity of the encapsulated sample.
433 The use of maltodextrin as wall material was more appropriate for preserving these
434 components providing the highest retention percentages of phenolic compounds and
435 flavonoids within the matrix and also the best functional properties for the encapsulated
436 sample, especially when freeze-drying was performed. Finally, freeze-drying using
437 maltodextrin as coating material can be considered a good option for encapsulation of
438 antioxidant phenolic compounds extracted from spent coffee grounds since is able to retain
439 62% and 73% of phenolic compounds and flavonoids, respectively, preserving 73-86% of the
440 antioxidant activity existent in the original extract.
441
442 **Conflict of interest**
443 The authors declare that there is no conflict of interest.
444
445 **Acknowledgements**

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References

Arribas-Lorenzo, G., & Morales, F.J. (2010). Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. *Food and Chemical Toxicology*, *48*, 644-649.

Ballesteros, L.F., Cerqueira, M.A., Teixeira, J.A., & Mussatto, S.I. (2015). Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment. *Carbohydrate Polymers, 127,* 347-354.

Ballesteros, L.F., Ramirez, M.J., Orrego, C.E., Teixeira, J.A., & Mussatto, S.I. (2017). Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds. *Journal of Food Engineering*, *199*, 1-8.

Ballesteros, L.F., Teixeira, J.A., & Mussatto, S.I. (2014a). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and Bioprocess Technology, 7,* 3493-3503.

Ballesteros, L.F., Teixeira, J.A., & Mussatto, S.I. (2014b). Selection of the solvent and extraction conditions for maximum recovery of antioxidant phenolic compounds from coffee silverskin. *Food and Bioprocess Technology, 7,* 1322-1332.

469 Beckman, C.H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm
470 formation in wilt disease resistance and in general defence responses in plants?
471 *Physiological and Molecular Plant Pathology, 57,* 101-110.
472 Castro-Cabado, M., Casado, A., & San Román, J. (2016). Bio-based thermosets: Effect of the
473 structure of polycarboxylic acids on the thermal crosslinking of maltodextrins. *European*
474 *Polymer Journal, 78,* 91-105.
475 Ceballos, A.M., Giraldo, G.I., & Orrego, C.E. (2012). Effect of freezing rate on quality
476 parameters of freeze dried soursop fruit pulp. *Journal of Food Engineering, 111*, 360-365.
477 Cilek, B., Luca, A., Hasirci, V., Sahin, S., & Sumnu, G. (2012). Microencapsulation of
478 phenolic compounds extracted from sour cherry pomace: effect of formulation,
479 ultrasonication time and core to coating ratio. *European Food Research and Technology*
480 *235*, 587-596.
481 Cho, A.-S., Jeon, S.-M., Kim, M.-J., Yeo, J., Seo, K.-I., Choi, M.-S., & Lee, M.-K. (2010).
482 Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat
483 diet-induced-obese mice. *Food and Chemical Toxicology, 48*, 937-943.
484 Conde, C., & Mussatto, S.I. (2016). Isolation of polyphenols from spent coffee grounds and
485 silverskin by mild hydrothermal pretreatment. *Preparative Biochemistry & Biotechnology,*
486 *46*, 406-409.
487 Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols–a review. *Trends in Food*
488 *Science & Technology, 21*, 510-523.
489 Farah, A., & Donangelo, C.M. (2006). Phenolic compounds in coffee. *Brazilian Journal of*
490 *Plant Physiology, 18*, 23-36.
491 Fernandes, R.V.B., Borges, S.V., & Botrel, D.A. (2014). Gum
492 arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary
493 essential oil. *Carbohydrate Polymers, 101*, 524-532.

494 Flores, F.P., Singh, R.K., Kerr, W.L., Pegg, R.B., & Kong, F. (2014). Total phenolics content
495 and antioxidant capacities of microencapsulated blueberry anthocyanins during in vitro
496 digestion. *Food Chemistry, 153*, 272-278.
497 Gouin, S. (2004). Microencapsulation: industrial appraisal of existing technologies and trends.
498 *Trends in Food Science & Technology, 15*, 330-347.
499 International Programme on Chemical Safety – IPCS (1999). Furfural. Prepared by the Fifty-
500 first meeting of the Joint FAO/WHO. Expert Committee on Food Additives (JECFA).
501 Geneva. Available at: http://www.inchem.org/documents/jecfa/jecmono/v042je03.htm
502 (Accessed 25/05/2017).
503 Karthikesan, K., Pari, L., & Menon, V.P. (2010). Antihyperlipidemic effect of chlorogenic
504 acid and tetrahydrocurcumin in rats subjected 1to diabetogenic agents. *Chemico-*
505 *Biological Interactions, 188*, 643-650.
506 Kasai, H., Fukada, S., Yamaizumi, Z., Sugie, S., & Mori, H. (2000). Action of chlorogenic
507 acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation in
508 vitro and in a rat carcinogenesis model. *Food and Chemical Toxicology, 38*, 467-471.
509 Lavelli, V., Harsha, P. S., & Spigno, G. (2016). Modelling the stability of maltodextrin-
510 encapsulated grape skin phenolics used as a new ingredient in apple puree. *Food*
511 *chemistry*, 209, 323-331.
512 Leonor, S.J., Gómez, J.A., Kinoshita, A., Calandreli, I., Tfouni, E., & Baffa, O. (2013). ESR
513 spectroscopic properties of irradiated gum Arabic. *Food Chemistry, 141*, 1860-1864.
514 Mahdavee Khazaei, K., Jafari, S.M., Ghorbani, M., & Hemmati Kakhki, A. (2014).
515 Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's
516 anthocyanins and evaluating their storage stability and color. *Carbohydrate Polymers,*
517 *105*, 57-62.

518 Mehanna, N.S., Hassan, Z.M.R., El-Din, H.M.F., Ali, A.A.-E., Amarowicz, R., & El-Messery,
519 T.M. (2014). Effect of Interaction phenolic compounds with milk proteins on cell line.
520 *Food and Nutrition Sciences, 5,* 2130.
521 Meneses, N.G.T., Martins, S., Teixeira, J.A., & Mussatto, S.I. (2013). Influence of extraction
522 solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains.
523 *Separation and Purification Technology, 108,* 152-158.
524 Murkovic, M., & Pichler, N. (2006). Analysis of 5-hydroxymethylfurfual in coffee, dried
525 fruits and urine. *Molecular Nutrition & Food Research*, *50*, 842-846.
526 Murthy, P.S., & Naidu, M.M. (2012). Recovery of phenolic antioxidants and functional
527 compounds from coffee industry by-products. *Food and Bioprocess Technology, 5,* 897-
528 903.
529 Mussatto, S.I. (2015). Generating biomedical polyphenolic compounds from spent coffee or
530 silverskin. In V.R. Preedy (Ed.), *Coffee in Health and Disease Prevention* (pp. 93-106).
531 London: Elsevier.
532 Mussatto, S.I., Machado, E.M.S., Martins, S., & Teixeira, J.A. (2011a). Production,
533 composition and application of coffee and its industrial residues. *Food and Bioprocess*
534 *Technology, 4*, 661-672.
535 Mussatto, S.I., Ballesteros, L.F., Martins, S., & Teixeira, J.A. (2011b). Extraction of
536 antioxidant phenolic compounds from spent coffee grounds. *Separation and Purification*
537 *Technology, 83,* 173-179.
538 Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of
539 encapsulation technologies for food applications. *Procedia Food Science, 1*, 1806-1815.
540 Paini, M., Aliakbarian, B., Casazza, A.A., Lagazzo, A., Botter, R., & Perego, P. (2015).
541 Microencapsulation of phenolic compounds from olive pomace using spray drying: A
542 study of operative parameters. *LWT - Food Science and Technology, 62*, 177-186.

543 Pandey, K., & Theagarajan, K. (1997). Analysis of wood surfaces and ground wood by
544 diffuse reflectance (DRIFT) and photoacoustic (PAS) Fourier transform infrared
545 spectroscopic techniques. *Holz als Roh-und Werkstoff, 55*, 383-390.
546 Panusa, A., Zuorro, A., Lavecchia, R., Marrosu, G., & Petrucci, R. (2013). Recovery of
547 natural antioxidants from spent coffee grounds. *Journal of Agricultural and Food*
548 *Chemistry, 61,* 4162-4168.
549 Paulino, A.T., Guilherme, M.R., Mattoso, L.H., & Tambourgi, E.B. (2010). Smart hydrogels
550 based on modified gum arabic as a potential device for magnetic biomaterial.
551 *Macromolecular Chemistry and Physics, 211,* 1196-1205.
552 Ramírez, M.J., Giraldo, G.I., & Orrego, C.E. (2015). Modeling and stability of polyphenol in
553 spray-dried and freeze-dried fruit encapsulates. *Powder Technology, 277*, 89-96.
554 Ray, S., Raychaudhuri, U., & Chakraborty, R. (2016). An overview of encapsulation of active
555 compounds used in food products by drying technology. *Food Bioscience, 13*, 76-83.
556 Reda, S.Y. (2011). Evaluation of antioxidants stability by thermal analysis and its protective
557 effect in heated edible vegetable oil. *Food Science and Technology, 31*, 475-480.
558 Ribeiro, J.S., Salva, T.J., & Ferreira, M. (2010). Chemometric studies for quality control of
559 processed Brazilian coffees using DRIFTS. *Journal of Food Quality, 33,* 212-227.
560 Rosa, C.G., Borges, C.D., Zambiazi, R.C., Rutz, J.K., Luz, S.R., Krumreich, F.D., Benvenutti,
561 E.V., & Nunes, M.R. (2014). Encapsulation of the phenolic compounds of the blackberry
562 (*Rubus fruticosus*). *LWT - Food Science and Technology, 58,* 527-533.
563 Saavedra-Leos, Z., Leyva-Porras, C., Araujo-Díaz, S.B., Toxqui-Terán, A., & Borrás-
564 Enríquez, A.J. (2015). Technological application of maltodextrins according to the degree
565 of polymerization. *Molecules, 20,* 21067-21081.
566 Santiago-Adame, R., Medina-Torres, L., Gallegos-Infante, J.A., Calderas, F., González-
567 Laredo, R.F., Rocha-Guzmán, N.E., Ochoa-Martinez, L.A., & Bernad-Bernad, M.J.

(2015). Spray drying-microencapsulation of cinnamon infusions (*Cinnamomum zeylanicum*) with maltodextrin. *LWT - Food Science and Technology, 64,* 571-577.

Scientific Committee on Consumer Safety – SCCS (2012). Opinion of Furfural. The SCCS adopted this opinion at its 14th plenary meeting of 27 March 2012. Available at: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_083.pdf (Accessed 25/05/2017).

Shin, H.S., Satsu, H., Bae, M.-J., Zhao, Z., Ogiwara, H., Totsuka, M., & Shimizu, M. (2015). Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in C57BL/6 mice. *Food Chemistry, 168,* 167-175.

Silva, F.C., Fonseca, C.R., Alencar, S.M., Thomazini, M., Carvalho Balieiro, J.C., Pittia, P., & Favaro-Trindade, C.S. (2013). Assessment of production efficiency, physicochemical properties and storage stability of spray-dried propolis, a natural food additive, using gum Arabic and OSA starch-based carrier systems. *Food and Bioproducts Processing, 91,* 28-36.

Zuorro, A., & Lavecchia, R. (2012). Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *Journal of Cleaner Production, 34,* 49-56.

593 **Figure captions**

594

595 **Fig. 1.** Chromatogram profile of the extract obtained by autohydrolysis of spent coffee
596 grounds (SCG) (a). X-ray diffractogram (XRD) (b), Fourier transform infrared spectra (FTIR)
597 (c), thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC) curves (d)
598 of the extract obtained by autohydrolysis of SCG and then precipitated with ethyl acetate.

599

600 **Fig. 2.** Scanning electron micrographs (SEM) for pure maltodextrin and gum arabic, as well
601 as for the phenolic compounds encapsulated and dried by freeze-drying and spray-drying.
602 Magnification, 2,500-fold.

603

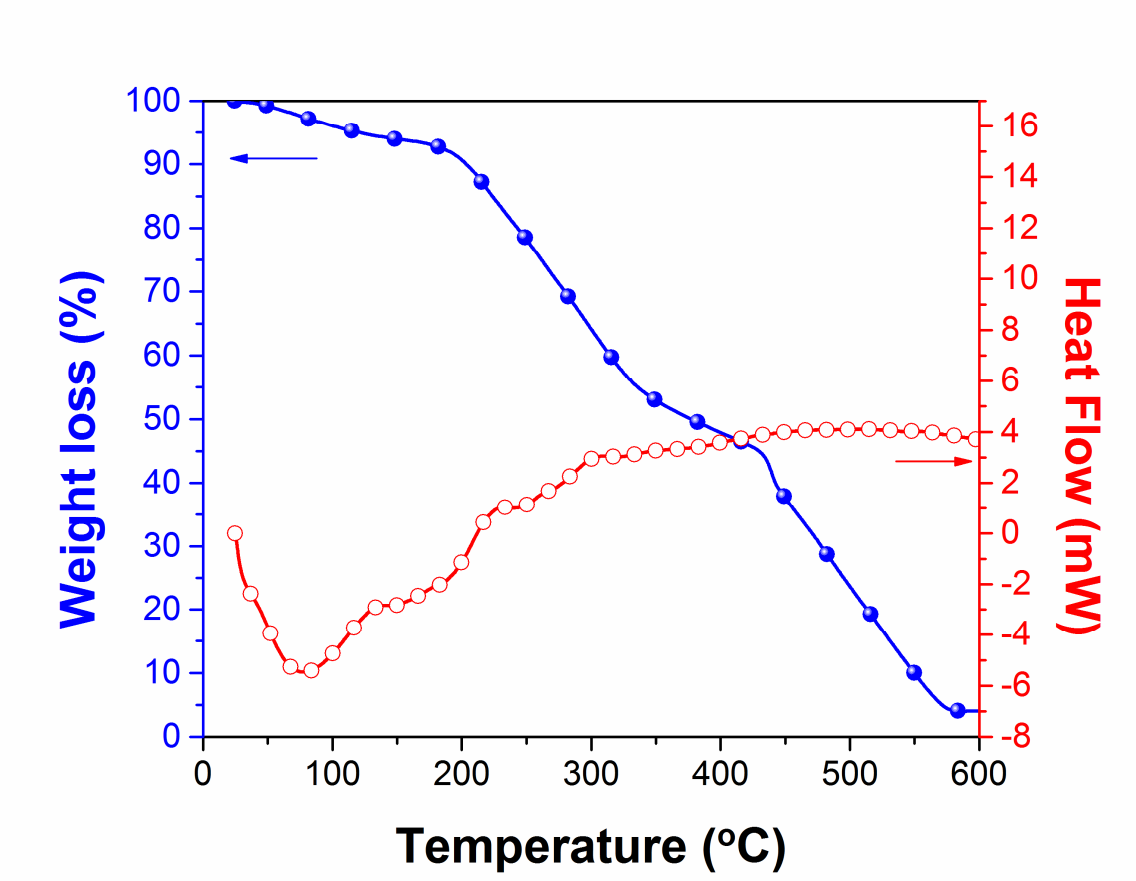
604 **Fig. 3.** X-ray diffractogram (XRD) (a) and Fourier transform infrared spectra (FTIR) (b)
605 obtained for pure maltodextrin and gum arabic, as well as for the phenolic compounds
606 encapsulated by freeze-drying and spray-drying. FWHM: full width at half maximum.

607

608 **Fig. 4.** Thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC)
609 curves for pure maltodextrin and gum arabic, and for the samples of spent coffee grounds
610 extract encapsulated into these coating materials, dried by freeze-drying and spray-drying.

611

612 **Fig. 5.** Percentage of encapsulated compounds taking into account their initial amount present
613 in SCG extract and their final amount retained into the coating materials, dried by freeze-
614 drying and spray-drying. Different letters within each method (PC: phenolic compounds;
615 FLA: flavonoid content; FRAP: antioxidant activity by the ferric reducing antioxidant power
616 assay; TAA: antioxidant activity by the total antioxidant activity assay) mean values
617 statistically different at 95% confidence level.



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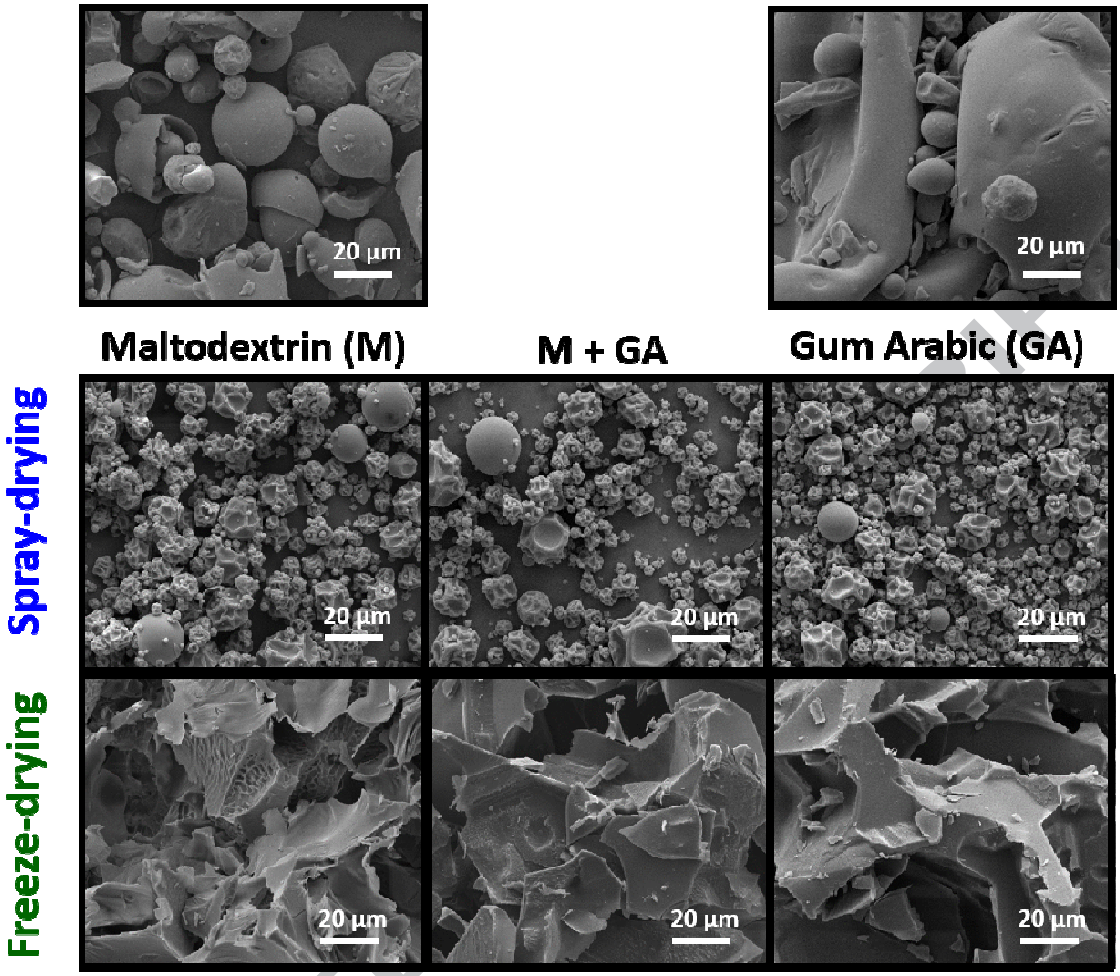

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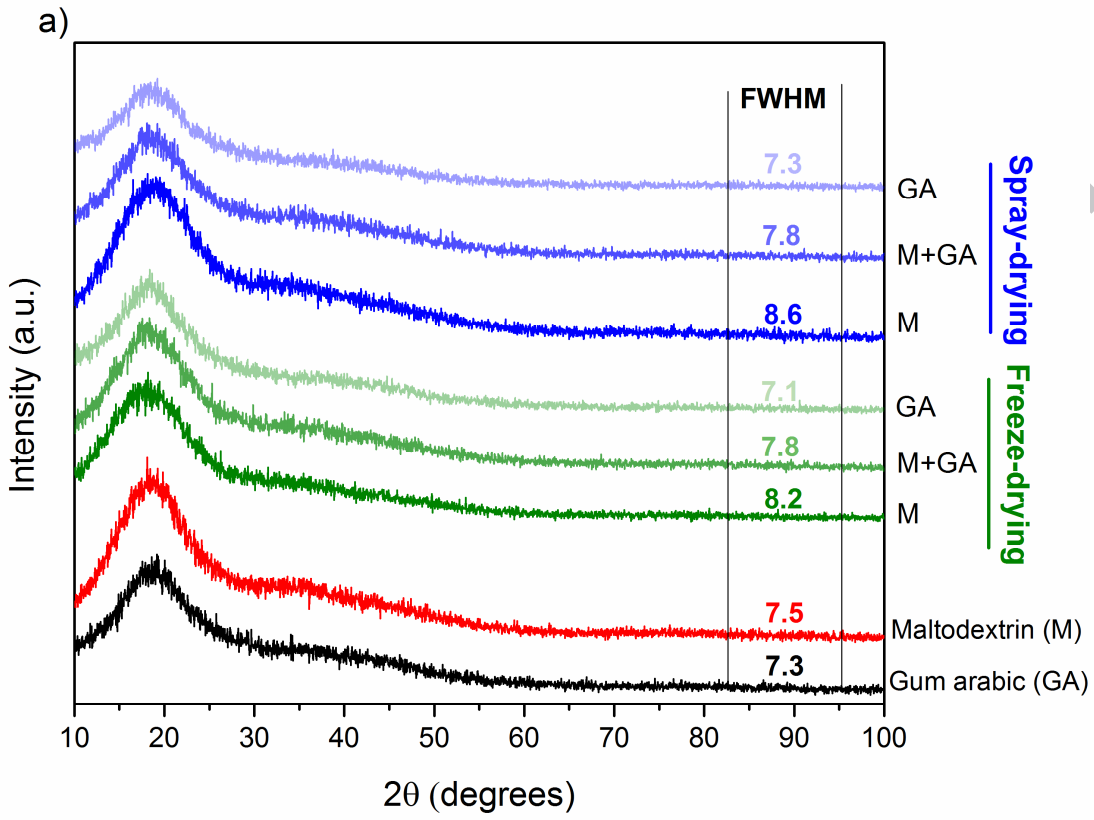

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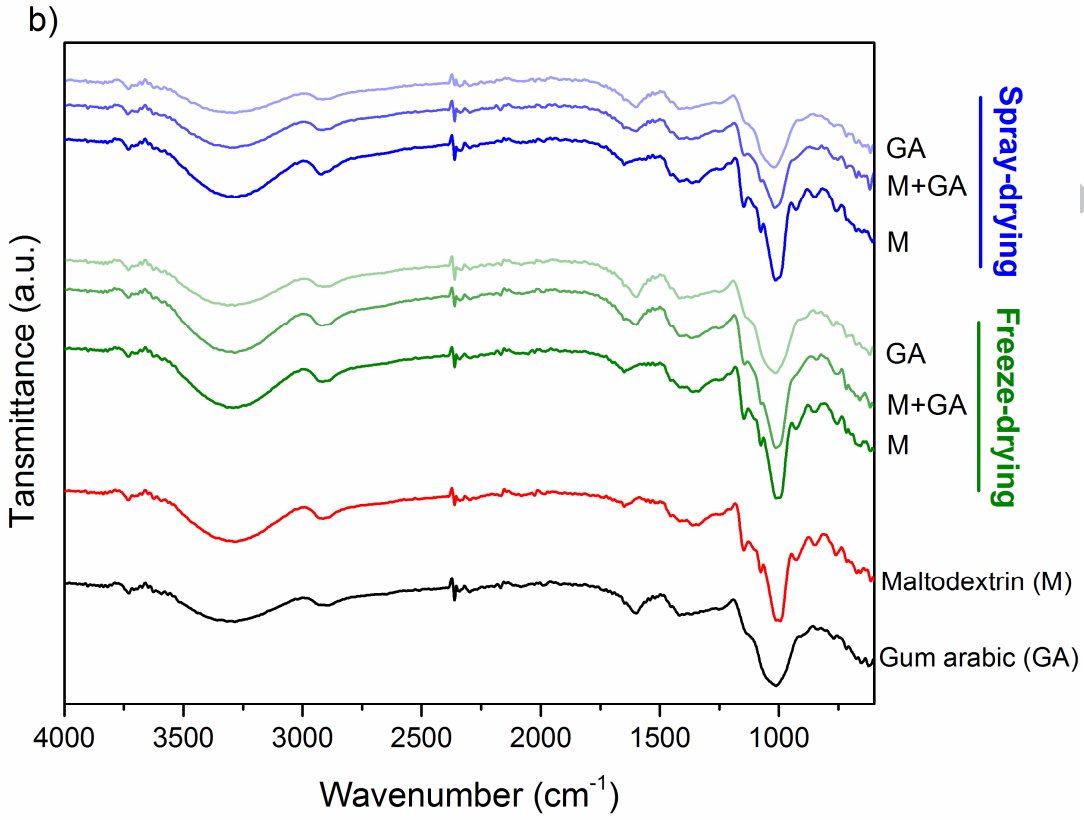

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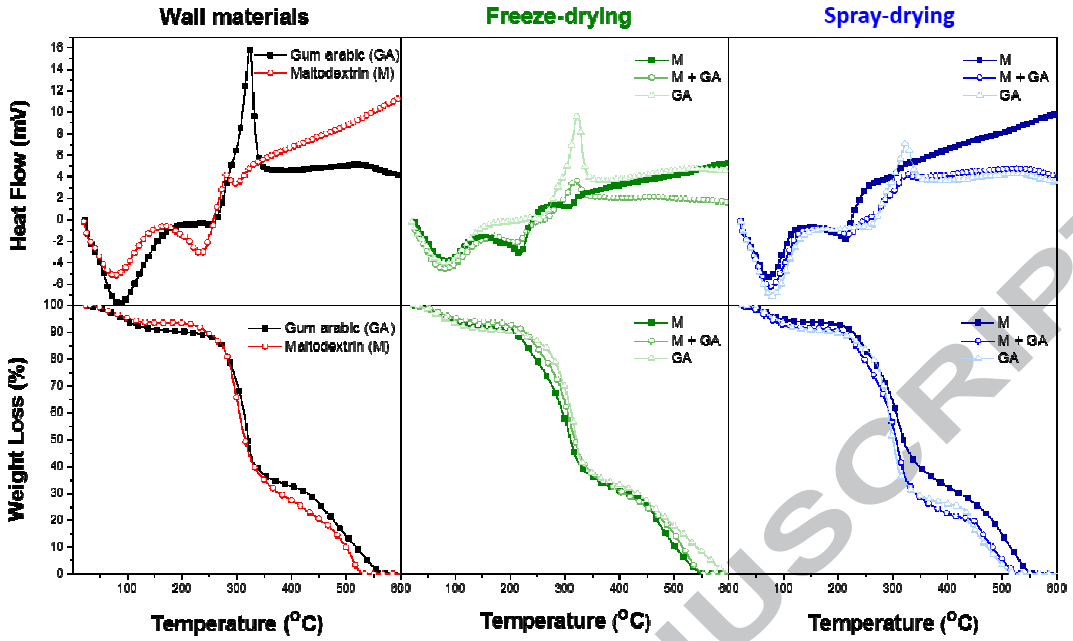

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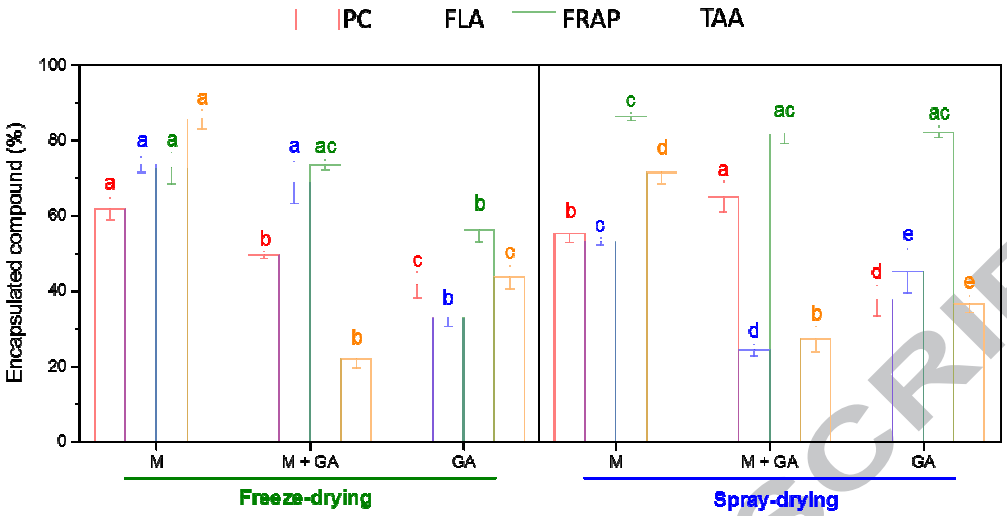

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Table 1

Contents of phenolic compounds and flavonoids and antioxidant activity of the extract produced from spent coffee grounds (SCG) before and after encapsulation into different coating materials by freeze-drying or spray-drying.

Drying process	Sample	PC (mg GAE/100 ml)	FLA (mg QE /100 ml)	FRA (mm
	SCG Extract	350.28 ± 11.71	16.51 ± 1.03	2.15
Freeze-drying	M	216.37 ± 10.32	12.14 ± 0.34	1.56
	M + GA	173.57 ± 3.40	11.36 ± 0.93	1.58
	GA	145.32 ± 12.08	5.38 ± 0.33	1.21
Spray-drying	M	174.07 ± 7.27	7.88 ± 0.16	1.67
	M + GA	204.86 ± 13.00	3.60 ± 0.23	1.58
	GA	117.67 ± 12.58	6.72 ± 0.87	1.59

Results are expressed as mean ± standard deviation; n=6.

M: maltodextrin; GA: gum arabic; PC: phenolic compounds; FLA: flavonoids; FRAP: antioxidant activity by the ferric reducing antioxidant power assay; TAA: antioxidant activity by the total antioxidant activity assay.

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654 **HIGHLIGHTS**

655
656

657 Encapsulation of antioxidant phenolic compounds (PC) extracted from SCG
658 was studied The technique and the coating material greatly influenced the
659 encapsulation results Freeze-drying using maltodextrin as coating material
660 provided the best results
661 62% of PC present in the original extract were retained in the
662 encapsulated sample 73-86% of the antioxidant activity existent in the
663 original extract was preserved

664